HYBRIDIZATION BETWEEN TWO SPECIES OF SWALLOWTAILS, MEIOSIS MECHANISM, AND THE GENESIS OF GYNANDROMORPHS

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ABSTRACT. Hybridization between *Papilio machaon* L. and *P. polyxenes asterius* Stoll was carried out over four generations by backcrossing black female F_1 and further hybrids with wild *machaon* males. A bilateral gynandromorph (symmetrical mosaic for the black-yellow phenotype) was obtained. In the fourth generation, one brood from a single female had negligible mortality but yielded an abnormal sex ratio opposite that predicted by Haldane's Rule (45 males/86 females, ca. 1:2). The black-yellow character followed a perfect 1:1 segregation. Reexamination of previous data suggests that meiosis in Lepidoptera follows an unusual pattern: the sister chromatids segregate during the first division, and crossing over is frequently absent in females. Bilateral gynandromorphs are generally due to fertilization of binucleate oocytes. Segregation during the first meiotic division also can explain the patterns of gynandromorphs arising as autosomal mosaics, such as those described here.

Additional key words: *Papilio machaon, P. polyxenes asterius*, Papilionidae, sex ratio.

Hybridization between *Papilio machaon* L. from Europe and Japan and *P. polyxenes asterius* Stoll from the United States was first undertaken more than 20 years ago (Clarke & Sheppard 1953, 1955, Ae 1966). Commercial availability of pupae of both species has allowed many breeders to easily carry out this cross since then. We recently performed such crosses, and our results are peculiar. Moreover, a spectacular gynandromorph emerged from one of our broods, and we compare this specimen with other examples recently described by Clarke and Clarke (1983).

MATERIALS AND METHODS

Papilio polyxenes asterius stocks were established from diapausing pupae obtained from Chicago, Cook Co., Illinois, and we obtained *P. machaon* from Cahors, Lot, France. The insects were hand-paired using the technique of Clarke (1952). Mated females were fed with a honey-water mixture (1:10), and allowed to oviposit in a gauze cage on carrot (*Daucus*) leaves. Either sunshine or light of a 60 W bulb at a distance of 20 cm was used to activate the insect. Number of ova laid per female

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was between 50 and 100, sometimes more. Larvae were reared on carrot leaves, or occasionally on other Umbelliferae. While no pathology was noticed in former broods, it has inhibited breeding in recent years. This fact may be related to extensive use in our neighborhood of the insecticide "Bactospéine", which contains strains of *Bacillus thuringiensis* Berl. Many diseased pupae and adults showed teratological atrophies comparable to those induced by toxin of this bacterium (Burgerjon & Biache 1967). Similar abnormalities also have been observed in pure strains of *Papilio polyxenes asterius* bred in the United States (Carter & Feeny 1985).

We encountered difficulties obtaining functional males in our breeding stocks, even among non-hybrids. Thus, hybrid females were always used, while the males were pure *machaon* from wild stocks. Crosses were performed over four generations.

RESULTS

Several broods comprised the F_1 generation ($\female$ *asterius* × $\male$ *machaon*), and gave the same results as those of Clarke and Sheppard's (1953, 1956) experiments: each offspring was as melanic as *asterius* and the anal eye-spot was intermediate. F_2 backcrosses ($\female F_1$ × $\male$ *machaon*) gave the expected 1:1 segregation between "black" and "yellow". In one brood, a remarkable gynandromorph was obtained (Figs. 1, 2). It is bilateral, with all of the left side being female with a "black" phenotype, and the eye-spot very close to *machaon*. The underside, although melanic, shows a strong *machaon* influence in distal parts of the wing. These features are characteristic of this kind of backcross. Markedly smaller, the right side is mainly male, and extremely *machaon*-like. However, on the hindwing, a melanic patch is present in the anal part. Its shape is complex, and its anterior border coincides with a compartment limit (Sibatani 1983) in the middle of the cell. The body is conspicuously halved in "black" and "yellow".

The third and fourth generations were obtained by pairing melanic females from the previous backcross with wild male *machaon*. As noted by Clarke and Sheppard (1956), fertility gradually increased. In the fourth generation, we were fortunate to obtain a large, healthy brood from a single female: 131 adults from 135 ova. Among them, 65 were of the "yellow" phenotype (18 males, 48 females) and 66 of the "black" (27 males, 38 females). Therefore, if the "yellow"/"black" ratio of 65/66 is truly 1:1, the sex ratio is strongly distorted (45/86, $\chi^2 = 12.83$, $P < 0.001$). The latter proportion is close to a 1:2 ratio. The cross presents another intriguing feature: the excess of females is more marked in the "yellow" phenotype than in the "black", where it does not even reach a significant level (27/38, $\chi^2 = 1.86$, P < 0.2). Analysis of these data

FIGS. 1, 2. *P. machaon* × *P. polyxenes asterias* F_2 hybrid, bipartite mosaic and gynandromorph. **1**, Dorsal surface; **2**, Ventral surface. Shown at ⅔ natural size.

through a contingency table indicates that this abnormality of distribution is on the borderline of significance ($\chi^2 = 2.955$, $0.10 > P > 0.05$).

A second gynandromorph arose in another brood of the fourth generation; it is a "mosaic", with the same "black" phenotype throughout. Gynandromorphism is apparent only in the parts where the male differs from the female. This specimen resembles closely those described by Clarke et al. (1977). No element of symmetry could be observed in this individual.

DISCUSSION

Some gynandromorphs of *Papilio* have already been described. A discussion once arose in the *News of the Lepidopterists' Society* about interpretation of gynandromorphs of *Papilio glaucus* L. (Walsten 1977, Silberglied 1977); a reanalysis of these examples was provided by Clarke and Clarke (1983). We next review some problems raised by sex genetics and the origin of gynandromorphs in Lepidoptera.

Morgan and Bridges (1919) showed that, in *Drosophila*, gynandromorphism is due to an irregular disjunction of sex chromosomes, leading to the loss of an "X" in one of the daughter cells. So, one-half of the organism would bear an "XX" set and would be female, and the other an "XO" and would be male (the Y chromosome is considered to bear very little information in this insect). In only one case in Lepidoptera has this mechanism been conclusively demonstrated, in the moth *Abraxas grossulariata* (Morgan & Bridges 1919), but Clarke and Clarke (1983) consider it a very likely explanation in some other cases. In most other examples, another mechanism seems to be involved: fertilization of a binucleate oocyte, as explained by Goldschmidt (1931). During meiosis,
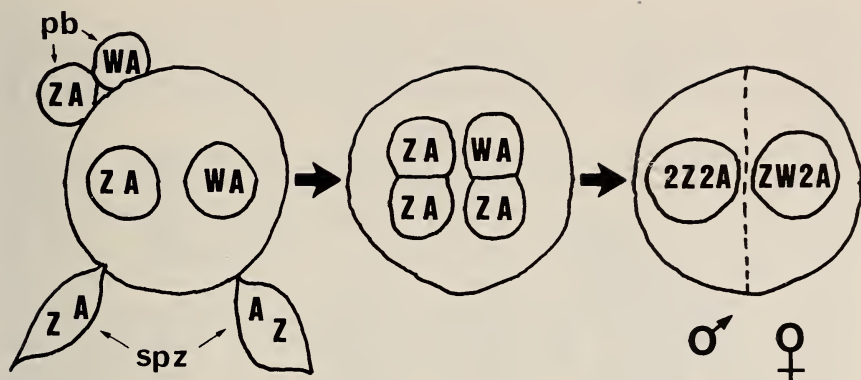
FIG. 3.   Schematic presentation of the mechanism producing gynandromorphs from a binucleate oocyte. Z, W: sex chromosomes; A: autosomal stock. pb: polar bodies from the former division; spz: the two spermatozoa which will fertilize both female pronuclei.

the two successive divisions lead to four haploid nuclei. Normally, three of them are eliminated. In some cases, which appear to be scarce, but whose frequency may be increased by certain mutations, two nuclei remain in the central zone of the oocyte, and both become fertilized. There follows a juxtaposition of the two eggs, which may have a genetic composition as different as any combination of two brothers and sisters. Figure 3 illustrates this phenomenon; we use "Z" and "W" for hetero-chromosomes, ZZ being male and ZW female. This phenomenon has been observed and photographed by Goldschmidt and Katsuki (1927).

As Robinson (1971) pointed out, this mechanism raises problems related to chromosome segregation in meiosis. Meiosis may proceed in two ways:

1) Sister chromatids issuing from the same single parental one may separate in the first mitosis of meiosis; the second mitosis therefore dissociates mother- and father-issuing homologous chromosomes.

2) The first mitosis separates mother- and father-issuing sets of chromosomes, and the second one, the sister chromatids.

The second way is considered normal in animals and plants. Of course, division of the centromere is expected to play a key role in this phenomenon. Actually, it is very difficult to observe the process cytologically and to demonstrate it genetically. It is only in oocytes that daughter cells undergo such a dissimilar fate.

The study of gynandromorphs and mosaics originating from binucleate oocytes may provide a clue to the precise order of chromatid segregation. When such abnormalities arise, they are most likely due to the two pronuclei issuing from the second mitosis remaining in the

middle of the oocyte. Gynandromorphs and bipartite mosaics can arise only if chromosome sets present in each symmetrical fertile pronuclei are genetically different. This implies that meiosis follows the first way above.

Cockayne (1935) clearly showed that there are two types of respective segregation in autosomes and sex chromosomes. 1) In *Bombyx mori*, gynandromorphs and uni- or bisexual mosaics are observed with the same frequency (Goldschmidt & Katsuki 1927). This shows that the two fertile pronuclei may either be both "Z" or both "W", or one "Z" and one "W", and it means that the chromosomes are segregating at random, likely due to an achiasmatic meiosis. 2) In *Argynnis paphia*, Goldschmidt and Fischer (1927) studied a strain where gynandro-morphism occurred regularly, probably because of a mutation produc-ing abnormal meiosis. In some cases, the autosomal and sex-conditioned mutant "*valesina*" was involved in the crosses. In contrast with *Bombyx*, "*valesina*"-normal mosaics are only observed when there is also gyn-andromorphism, and no unisexual mosaic occurs in this strain. The unambiguous conclusion (not stated by Goldschmidt and Fischer or Cockayne) is that always, when a binucleate oocyte is formed, one pronucleus bears a "Z" and the other a "W"—a strong argument in favor of meiosis with preliminary separation of sister chromatids. In the sphingid *Laothoe populi*, and in many other instances, things appear identical. An illustrative example was recently provided by Platt (1983), in artificial hybrids of *Limenitis arthemis* and *L. lorquini*; he also interpreted the bipartite mosaic-gynandromorph he obtained by the "double egg" theory. However, we are reminded of a halved "*alba*"-orange female of *Colias croceus* figured by Frohawk (1938); since various kinds of gynandromorphs have been described in this species (including "*alba*" female-orange male), this case might rather corre-spond to the silkworm type; however, mosaics may arise from various causes and, isolated, this record remains inconclusive.

Previous paragraphs deal only with bipartite gynandromorphs and mosaics; however, most sexual mosaics are asymmetrical, which can be explained in two ways: either, in the case of binucleate oocytes, one pronucleus becomes shifted from the central region of the oocyte, or sex chromosomes segregate abnormally during further division of em-bryonic cells. This latter event most likely explains the minute patches which characterize the bulk of so-called gynandromorphs.

The first gynandromorph described in this study fits perfectly with the double-oocyte theory, and supports the assumption that meiosis obeys the first-named way in *Papilio*. In the double oocyte that gave rise to this individual, the left pronucleus was "W" and "black" and the right one "Z" and "yellow" (Fig. 4). The only puzzling point comes
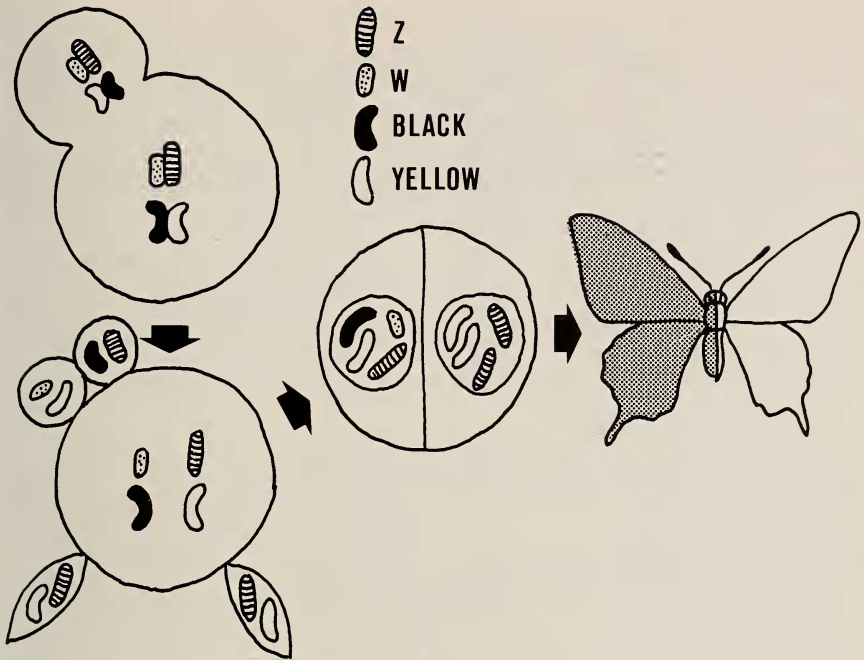
FIG. 4.   Scheme of the mechanism leading to the gynandromorph of Figs. 1, 2. Only the color character-bearing autosome pair is figured.

from the small melanic patch on the right hindwing. It is inadmissible that it arose from a chromosome loss, since "black" is dominant and could not be present in the genetic stock of the right half. Thus it should be due either to presence of an uneliminated third pronucleus, remnant from the first division of meiosis, or to an erratic cell coming from the left half. The second gynandromorph may be best explained by an atypical segregation of sex chromosomes during embryogenesis. If it arose from a binucleate oocyte, this would imply that achiasmatic meiosis could occur in *Papilio* as in *Bombyx*.

According to Suomalainen (1965), the first-named type of meiosis is determined by the holocentric nature of centromeres, which he has indeed observed in Lepidoptera; he states also that, in this order, no crossing over occurs in the female sex. These assumptions have suffered controversy from Robinson (1971) and White (1973) as remaining undemonstrated in the whole of Lepidoptera, but they have been firmly ascertained for some species, such as *Bombyx mori* (Tazima 1964), *Heliconius* (Turner & Sheppard 1975), *Anagasta kuehniella* (Traut 1977).

The abnormal sex ratio observed in one brood of the fourth generation

(which is corroborated by other less quantitative observations made at the same time) is most difficult to interpret. We have no definitive explanation to propose; we only offer remarks that may help future investigations. The distortion goes against Haldane's (1922) Rule. Here, it is the heterogametic sex that is favored; the ♂/♀ ratio is close to 1:2. It is not possible to explain this discrepancy by the death of one-half of the males, since the mortality from egg to adult was very low. We must invoke a possible abnormality during meiosis.

A non-disjunction of "Z" chromosomes during oogenesis could produce a sex ratio distortion in the direction observed. It should give rise to a proportion of 2 (W, A) oocytes, 1 (ZZ, A) and 1 (O, A). Hence, following fertilization, we should have 1/2 ZW, 2A normal females, 1/4 ZO, 2A females (such a formula is usually considered to correspond to females) and 1/4 ZZZ, 2A males. But should the two latter types of individuals display a viable phenotype? We could not detect any abnormality in offspring of the concerned brood. Moreover, if generalized, this mechanism should give rise to 3/4 females, while we observed 45/86 ($\chi^2 = 6.5$, P < 0.01); therefore, non-segregation acted only partially.

Meiotic drive is another phenomenon which could lead to sex ratio distortion. This is a preferential segregation of certain chromosomes in functional gametes (detailed review in Zimmering et al. 1970). Recent data indicate that this phenomenon is rather widespread, and involves a higher frequency sex and "B" (heterochromatine) chromosomes. Sex chromosomes differ in both of our species by a heterochromatic segment present only in *machaon*. Moreover, the "W" from *asterius* does not pair perfectly with the "Z" from either species (Clarke et al. 1977). Do these peculiarities trigger meiotic drive preferentially directing the "Z" towards a polar body? Such a phenomenon would produce normal karyotypes; being unaware of the problem, we did not check karyotypes.

Moreover, we should consider that sex ratio distortion perhaps affects the "yellow" phenotype somewhat more; this could mean that there is "attraction" between the *asterius*-originated "W" and the color-controlling autosome which comes from *machaon*. One possible explanation is that these two chromosomes possess certain sequences in common, and that they could pair, at least partly, during meiotic prophase. This should obviously affect further segregation, the color-bearing autosomes being the "drivers", as is indicated by their overall 1:1 proportion. Both species should therefore differ by a translocation between sex chromosomes and color-bearing autosomes. This hypothesis is not as fancy as it may appear at first sight, since in related American species such as *Papilio glaucus*, the color-controlling segment itself is carried on the "W" chromosome (Clarke & Clarke 1983).

CONCLUSION

Reexamination of previous data and analysis of the experiments presented here allow us to conclude that:

1) In butterflies, at least in the vast majority, meiosis obeys a rather unusual pattern where chromatid segregation follows an order opposite the normal one. This is also probably true for moths (the Silkworm case being the most extreme, since in its meiosis it is achiasmatic).

2) Bilateral gynandromorphs arise most often from fertilization of a double oocyte. The determinism of mosaic gynandromorphs is more complex and may result from completely different causes.

3) In *Papilio*, one can carry interspecific crosses over a large number of generations by using backcrosses, in a kind of "monitored introgression".

4) Even in *Papilio,* however, a residual amount of genetic incompatibility occurs. Sex chromosomes are the most sensitive to disturbances resulting from this incompatibility.

We surmise that some kind of abnormal chromosome segregation takes place in interspecific crosses, and we hope our findings will stimulate further research on these questions.

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